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# Comprehensive Analysis of a Norovirus-Associated Gastroenteritis Outbreak, from the Environment to the Consumer▽

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**Noroviruses have been recognized to be the predominant agents of nonbacterial gastroenteritis outbreaks in humans, and their transmission via contaminated shellfish consumption has been demonstrated. Norovirus laboratory experiments, volunteer challenge studies, and community gastroenteritis outbreak investigations have identified human genetic susceptibility factors related to histo-blood group antigen expression. Following a banquet in Brittany, France, in February 2008, gastroenteritis cases were linked to oyster consumption. This study identified an association of the norovirus illnesses with histo-blood group expression, and oyster contamination with norovirus was confirmed by qualitative and quantitative analyses. The secretor phenotype was associated with illness, especially for the non-A subgroup. The study showed that, in addition to accidental climatic events that may lead to oyster contamination, illegal shellfish collection and trading are also risk factors associated with outbreaks.**

Since they were first identified as the cause of a gastroenteritis outbreak in an elementary school in Norwalk, OH, in 1968, noroviruses (NoVs) have come to be recognized as important agents of nonbacterial gastroenteritis in humans (3). NoVs are small nonenveloped viruses containing a single-stranded, positive-sense RNA genome and constitute one of the six genera in the family *Caliciviridae*. On the basis of genomic sequence and phylogenetic analyses, the NoV genus contains more than 30 genetic types distributed into five genogroups, and they cause infection principally in humans but also in some animals (46). Since the end of the last century, genogroup II (GII) strains have predominated among humans, but numerous strains presenting genomic diversity cocirculate in the population. Many NoV strains bind to histo-blood group antigens (HBGAs) (40). HBGAs are complex glycans present on many cell types, including red blood cells and vascular endothelial cells, as well as on the epithelia of the gastrointestinal, urogenital, and respiratory tracts. HBGAs are synthesized from a series of precursor structures by the stepwise addition of monosaccharide units via a set of glycosyltransferases. In humans, the pleiotropic interaction of alleles at three loci, *FUT3*, *FUT2*, and *ABO*, determines the Lewis, secretor, and ABO phenotypes, respectively (28). The evidence that has accumulated from volunteers studies and from the analysis of outbreaks indicates that binding to these carbohydrates is required for infection (5, 6, 15, 17, 18, 25, 39). Moreover, various human NoV strains that bind to HBGAs present distinct specificities for HBGAs (13, 14, 38). As a result, most strains infect only a subset of the population, on the basis of HBGA expression (9, 24, 40). In addition, some strains of either GI or GII were shown to specifically attach to oyster tissues through the recognition of histo-blood group antigens (21, 30, 43, 44), suggesting that oysters may act as selective filters, specifically concentrating strains that can recognize carbohydrate epitopes shared with humans.

NoV infection is characterized by the sudden onset of vomiting or diarrhea, or both symptoms (3). Similar to other viruses causing gastroenteritis, NoVs multiply in the intestines and are excreted in large quantities in human feces. Human waste is processed in sewage treatment plants, but the treatment procedures do not completely remove enteric viruses from the water effluents leaving the plant (8, 16). Strains that cause severe symptomatic infections as well as those that cause subclinical infections are excreted into sewage, which may then be discharged into coastal environments (11). As these viruses are very resistant to inactivation, the sanitary consequences can include contamination of drinking water, vegetables, and bivalve molluscan shellfish (19). Mollusks such as oysters filter large volumes of water as part of their feeding activities and are able to accumulate and concentrate different types of pathogens. Regulations based on measurement of the levels of bacterial enteric pathogens in shellfish tissues (European regulation 54/2004/EC) or in water in which shellfish are grown (United States National Sanitation Program) have been instituted to protect consumers. However, despite these control measures, outbreaks linked to shellfish consumption still occur after either accidental contamination or incomplete depuration (22, 34, 45). Illegal shellfish collection and trading represent an additional source of food contamination that has re-

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ceived little attention so far. We report here on a norovirus outbreak that was due to a breach of such a regulation. In addition, quantitative data on oyster contamination and the number of oysters consumed in relation to the genetic susceptibility of exposed consumers are reported.

## MATERIALS AND METHODS

**Collection of epidemiological data and statistical analysis.** All data concerning cases of gastroenteritis were collected by the use of a standardized questionnaire that was completed by each participant and that addressed the foods consumed, the symptoms, and the timing of illness. Details on the patients and the course of the outbreak are presented in the Results section. The association between food consumption and illness was estimated by calculation of the relative risk (RR) and its 95% confidence interval (CI). Two-by-two comparisons between subgroups were performed by Fisher' exact test (two-tailed). Analysis of the association between the presence of symptoms and the number of oysters consumed was performed by the Wilcoxon signed-rank test. All statistical analyses were performed with the Prism (version 5) program (GraphPad Software Inc., La Jolla, CA).

**Analysis of clinical samples.** Five fecal samples collected from five patients were analyzed. Group A rotaviruses (RVs), astroviruses (AVs), and adenovirus (AdV) types 40 and 41 were detected as described previously (1). For confirmation, typing, and the detection of other enteric viruses, nucleic acids (NAs) were extracted and purified by using a QIAmp viral RNA kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. NoV GI and GII, sapoviruses (SaVs), hepatitis A virus (HAV), enteroviruses (EVs), and Aichi viruses (AiVs) were detected by the use of several reverse transcription-PCR (RT-PCR) assays that amplified regions previously demonstrated to be suitable for detection and/or typing (1).

**Analysis of shellfish samples.** Seven oyster samples (*Crassostrea gigas*) comprising at least 30 oysters per sample (except 18 oysters for sample 79) were collected. One sample (sample 75) was collected from leftovers at the caterer; three samples (samples 76, 77, and 78) were collected from an approved class A production area (less than 230 *Escherichia coli* cells/100 g of total flesh, according to European regulation 54/2004/EC), as identified by the REMI IFREMER Surveillance Network; one sample (sample 79) was collected from the producer depuration tank, within the same batch that caused illness; and the last two samples (samples 82 and 83) were collected from an area located 30 km from the approved production area and where the collection and trading of shellfish are illegal. The shellfish, which were kept at 4°C during shipment, were analyzed as described previously (4). Briefly, the stomach and digestive diverticula (DT) were removed by dissection (1.5-g portions), homogenized, extracted with chloroform-butanol, and treated with Cat-floc (Calgon, Ellwood City, PA). Virus was then concentrated by polyethylene glycol 6000 (Sigma, St. Quentin, France) precipitation (4). Viral NAs were extracted with a Nuclisens kit (bioMérieux, France), suspended in 100 μl of elution buffer with 20 U of RNase inhibitor (Invitrogen, France), and analyzed immediately or kept frozen at −80°C (23).

**Real-time RT-PCR.** All shellfish NA extracts were first screened by real-time RT-PCR (rRT-PCR) with previously published primers and probes for NoVs, HAV, AVs, and EVs (22). rRT-PCR was performed on an MX3000 detector (Stratagene, France) or an ABI Prism 7000 SDS detector (Applied Biosystems, France) with an Ultrasens one-step quantitative RT-PCR system (Invitrogen). All samples were analyzed in duplicate by the use of 5 μl of undiluted or 10-fold-diluted RNA extracts. Two negative amplification controls (water) were included in each amplification series, and no more than six samples were analyzed in an rRT-PCR assay. Precautions such as the use of isolated rooms for various steps and the use of filter tips were taken to prevent false-positive results.

The cycle threshold ($C_T$) was defined as the cycle at which a significant increase in fluorescence occurred (i.e., when the fluorescence became distinguishable from the background). The number of NoV RNA copies present in positive samples was estimated by using standard curves for GI and GII. To be included in the quantitative analysis, all wells had to yield a $C_T$ value of ≤41. The final concentration was then determined on the basis of the NA volume analyzed (5 μl of 100 μl of NA extract) and the measured weight of the DT (1.5 g was analyzed) (23).

The efficiency of the virus extraction procedures was determined for each extraction by seeding $10^4$ 50% tissue culture-infective doses of mengovirus prior to sample processing and determining the amount of mengovirus recovered by rRT-PCR, as described previously (7, 23). The NoV concentrations were then corrected for virus loss during extraction by dividing the final norovirus concentration (uncorrected) by the mean mengovirus extraction efficiency.

Evaluation for the presence of RT-PCR inhibitors was performed by the coamplification of 2.5 μl of each NA extract with 2.5 μl containing 100 copies of GI or GII RNA internal controls in separate experiments (23). The amplification of RNA indicated that no more than partial inhibition was present; no adjustments to the quantitative estimates were made for samples with partial inhibition.

**Standard RT-PCR.** The viruses that were detected in samples by rRT-PCR were typed by sequencing after amplification by use of a standard, two-step RT-PCR format and 40 cycles of amplification with the same primers used for the clinical samples (1, 22).

**Sequence analysis.** The amplicons from virus-positive samples were excised from the gel, extracted, and purified for sequencing by using a QIAex II gel extraction kit (Qiagen) (1). Sequencing with a BigDye Terminator cycle sequencing kit (Applera Corporation, Foster City, CA) was performed with the same primers used for amplification (1). The sequences were analyzed by comparison with the sequences in the European Food-Borne Viruses Database (https://hypocrates.rivm.nl/bnwww; FBVE QLK1-CT-1999-00594) for identification of the NoV genotypes. The sequences of the other enteric viruses were checked for homologies with sequences in the GenBank database by using the BLAST search program (April and May 2008).

**Phenotyping of saliva for ABO, secretor, and Lewis phenotypes.** Saliva samples were collected from 33 of 34 individuals who had eaten oysters and were immediately stored at −20°C. They were boiled prior to use in order to remove potentially contaminating virus-specific immunoglobulins as well as bacterial glycosidases. Phenotyping for the ABO, secretor, and Lewis characteristics was performed by enzyme-linked immunosorbent assay, as described previously, by using a set of monoclonal anti-A, anti-B, anti-H, and anti-Lewis antibodies and peroxidase-conjugated *Ulex europaeus* agglutinin I lectin (29).

## RESULTS

**Epidemiological investigation.** A banquet for lunch with 80 participants was organized on 5 February 2008 in Brittany, France. The participants were separated in two rooms, and oysters were served in only one room. All sick people had been seated in that room. The menu was a plate with three oysters, three langoustines, and five shrimps; fish and gratin dauphinois; cheese and salad; and fruit tart. As no sign of disease was found among the people who did not consume oysters, the epidemiological study rapidly implicated oysters.

A total of 34 people ate oysters, and the mean number of oysters consumed per person was 3.5 oysters (range, 2 to 6 oysters; 95% CI, 3.1 to 3.9 oysters). Twenty-three people got sick (Fig. 1), and the mean incubation period was 33.4 h (range, 8 to 50 h; 95% CI, 29.0 to 37.8 h). Vomiting was reported in 69% of cases, and diarrhea was reported in 87%. The duration of symptoms ranged from half a day up to 6 days, with the mean being 4.0 days (95% CI, 3.2 to 4.9 days). Two people visited a physician. The mean age of the cohort was 43.4 years (range, 23 to 60 years; 95% CI, 39.6 to 47.2 years).

**Results of stool analysis.** A total of five stool samples collected from five individual patients were analyzed. All stools were negative for AdVs, HAV, RVs, and EVs. No viral pathogen was detected from one stool sample (sample E2499). Three stool samples were positive for NoV GII, one sample of which (sample E2482) was also positive for SaV, and a fourth stool sample (sample E2455) was positive for AiV (Table 1). After sequencing, a GI.2 strain was identified for the SaV, three GII.4 strains and a GII.2 strain was identified for the NoVs, and a genotype A strain was identified for AiV.

**Results of shellfish analysis.** The first sample (sample 75), collected on 13 February 2008 in the restaurant from the batch that had been consumed, contained NoV GII RNA. The sample collected from the producer (sample 79) on 14 February and kept in a depuration tank for 23 days was also positive for

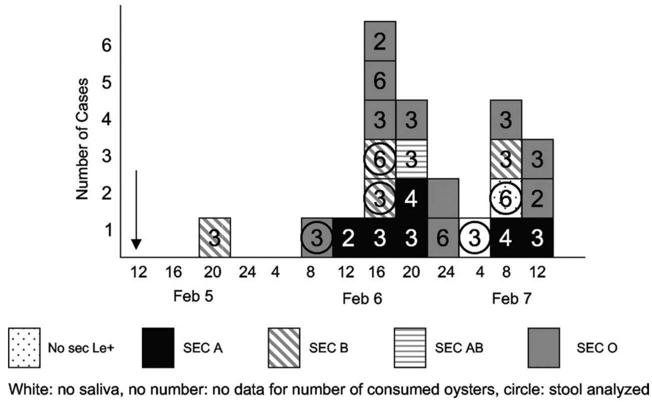


FIG. 1. Onset of symptoms for banquet participants. Oysters were consumed for lunch on 5 February 2008 (arrow). Each box represents one new clinical case identified in 4-h intervals (*x* axis), and the number of cases is recorded (*y* axis). The number in each box corresponds to the number of oysters consumed, and the circle identify people from whom stool samples were collected for analysis. Black boxes, type A secretors; gray boxes, type O secretors; box with horizontal stripe, a type AB secretor; boxes with diagonal stripes, type B secretors; boxes with dots, nonsecretors; white box, no saliva collected.

NoV GII as well as for SaV. Neither of these two samples was found to contain AV, AiV, EV, HAV, or RV. Three samples collected on 12 February from the producing area, located in South Brittany in a class A area (less than 230 *E. coli* cells/100 g of total flesh), were negative for all human enteric viruses evaluated. Two additional samples were collected on 25 February (sample 82) and 5 March (sample 83) from a distinct location that was presumptively linked to the outbreak (see Discussion). These two samples contained NoV GII, SaV, and AV but were negative for AiV, EV, RV, and HAV.

For quantitative analyses, positive samples 75, 82, and 83 were extracted four times, but sample 79 was extracted only three times due to the limited number of oysters available. The extraction efficiencies, as measured by the recovery of mengovirus seeded into the shellfish prior to sample processing, ranged from 12 to 16% for all extracts (Table 2). The NoV concentrations measured for samples 75 and 79 were quite variable, ranging from negative (one replicate among four samples for sample 75 and one negative sample among three samples for sample 79) to thousands of RNA copies per gram of DT (Table 2). The two other samples (samples 82 and 83) showed more homogeneous contamination for all replicates analyzed (all four replicates were positive). The geometric mean virus concentrations for all of these samples were quite similar.

**Sequence comparisons.** A short sequence (84 bp) in the NoV polymerase-coding region obtained for sample 75 corresponded to the GII.4 strain detected in two stool samples (samples E2457and E2458). The sequence obtained from oyster sample 82 was identical to that detected in stool sample E2482 over a 255-bp sequence amplified from the capsid-coding region. The sequence from sample 83 was identified as that of an NoV GII.3 strain (248 bp in the capsid region) and did not match that of any clinical case directly linked to the outbreak but was identical to a secondary case (the daughter of the provider of stool sample E2455). No virus sequence (NoV or SaV) could be obtained from sample 79.

**Association between expression of histo-blood group antigens and disease.** The ABO, Lewis, and secretor phenotypes for 33 individuals who had consumed oysters were determined by the use of saliva. The phenotyping gave clear-cut results for every case. The frequencies of the various phenotypes in this cohort did not differ from those in the French population (data not shown). Owing to the small number of Lewis phenotype-negative individuals (three among the secretors and one among the nonsecretors), the potential effect of the Lewis phenotype could not be analyzed.

No statistically significant associations were found between the ABO, secretor, and Lewis phenotypes and either the incubation time, the type of symptom (vomiting or diarrhea) that was reported, or the duration of the symptom(s) (data not shown). However, the frequency of individuals reporting illness was lower among nonsecretors than among secretors ($P < 0.01$, Fisher's exact test). When the individuals were evaluated by symptom, both vomiting and nausea were significantly less frequent in the nonsecretor group than in the secretor group. To analyze the effect of the ABO phenotype, the secretor group was split into the A, B, and O subgroups. A direct comparison of these three subgroups did not show any significant difference between them. However, compared to the nonsecretor group, only individuals in the B and O secretor groups showed a significantly higher frequency of illness (Table 3). The A secretor subgroup did not statistically differ from the nonsecretor group, indicating that most of the effect of the secretor phenotype was borne by the non-A subgroups (O and B). This suggests that the epithelial expression of the A blood group may have hindered recognition of the carbohydrate NoV receptor, and comparison of the A secretor group to the non-A secretor group (B and O secretor groups) indeed showed that among the secretors, A blood group individuals were less likely to have diarrhea ($P < 0.05$). This suggests that the A subgroup was not as sensitive as the non-A subgroup, although the small numbers of patients made it difficult for the difference to reach statistical significance.

Since the age of the subjects and the number of oysters consumed could be possible confounding factors, we verified that there was no association between any of the histo-blood group phenotypes and these two parameters (data not shown). When subjective symptoms, such as nausea and abdominal pain, are considered in addition to vomiting and diarrhea,

TABLE 1. Patient symptoms and viruses detected from stool samples

| Stool sample | Saliva phenotype[a] | Clinical signs | | Virus(es) detected | No. of oysters consumed[b] |
|---|---|---|---|---|---|
| | | Vomiting | Diarrhea | | |
| E2455 | SEC, B, Le | + | + | AiV | 6 |
| E2457 | SEC, B, Le | + | + | NoV GII.4[c] | 3 |
| E2458 | SEC, O, Le | + | + | NoV GII.4 | 3 |
| E2482 | No saliva | + | + | NoV GII.4, NoV GII.2, SaV GI.2 | 3 |
| E2499 | No sec, Le | − | + | None | 6 |

[a] SEC, secretor phenotype positive; B, B phenotype positive; Le, Lewis phenotype positive; O, O phenotype positive; No sec, nonsecretor.
[b] Number of oysters consumed during the lunch.
[c] The underlined GII.4 identifies strains with identical sequences detected in the oyster samples.

TABLE 2. Detection and quantification of NoV in oyster samples

| Sample source (sample no.) | Date (mo/day in 2008) | Mean extraction efficiency (%)[a] | No. of positive replicates/ no. analyzed | NoV GII geometric mean concn (range)[b] | |
|---|---|---|---|---|---|
| | | | | Uncorrected | Corrected |
| Caterer (75) | 02/13 | 16.1 | 3/4 | 229 (48–2,548) | 1,727 (89–23,162) |
| Producer (79) | 02/14 | 13.7 | 2/3 | 144 (63–273) | 957 (476–2,054) |
| Harvested illegally (82) | 02/25 | 12.9 | 4/4 | 301 (35–913) | 2,361 (273–7,509) |
| Forbidden area (83) | 03/05 | 12.6 | 4/4 | 776 (273–1,885) | 6,076 (211–14,839) |

[a] Mean percentage of mengovirus RNA copy number recovered in shellfish extracts relative to the amount of mengovirus added to the samples prior to extraction.
[b] Geometric mean concentration of positive samples expressed as the number of RNA copies per gram of DT (range of concentrations detected) before (uncorrected) and after (corrected) correction for the mean extraction efficiency.

more individuals in the cohort were affected. Interestingly, within the group of six nonsecretor individuals, the number of oysters consumed was higher among the three who reported at least one symptom ($P = 0.026$, Wilcoxon signed-rank test), suggesting that nonsecretors could not become infected below a certain virus dose threshold. These nonspecific symptoms of the nonsecretors were possibly due to infection with viruses other than norovirus. Alternatively, they could be due to the ability of the norovirus strain involved to show some cross-reactivity to carbohydrate motifs shared between secretors and nonsecretors, as previously observed for some strains (24, 41). Regardless, these results collectively indicate that the nonsecretor phenotype was a protective factor and that within the secretor group, blood group A was also protective.

## DISCUSSION

The institution of regulations to specify acceptable levels of bacterial enteric pathogens in shellfish tissues (European regulation 54/2004/EC) or in waters where shellfish are grown (United States National Shellfish Sanitation Program) led to the classification of production areas. In addition to mandatory controls performed by producers, in France, IFREMER has set up a surveillance network to control shellfish quality (REMI) and to collect data from environmental events. In the past, this allowed the identification and evaluation of contamination events associated with oyster-related outbreaks (20, 22). In the outbreak described here, the producing area was located in a class A area in South Brittany, and no environmental event such as rainfall or sewage treatment plant failure had been reported in the previous months. This was confirmed by the absence of viral contamination in the samples collected for the REMI controls on 12 February 2008. It was thus surprising to observe that both samples related to a specific producer from that clean class A area were clearly contaminated by viruses and were the cause of the clinical cases. A few days later, police arrested a fisherman illegally collecting oysters from an area located in a major harbor where oyster collection is forbidden. After a day in jail, the man admitted to selling these illegal oysters to the producer implicated in the outbreak. This was confirmed by sequencing of the NoV strains detected in the sample taken by the police (sample 82) and in the sample from the forbidden area (sample 83). This outbreak therefore clearly illustrates the danger of breaching regulations and refusing to consider the usefulness of the producing area classification, as some producers do.

TABLE 3. Effect of blood group phenotype on illness and symptom frequency

| HBGA | No. of individuals in the following group[a]: | | | | | |
|---|---|---|---|---|---|---|
| | Nonsec ($n = 6$) | Sec ($n = 27$) | A Sec ($n = 10$) | B Sec ($n = 4$) | O Sec ($n = 12$) | Non-A Sec ($n = 16$) |
| Illness[b] | 1 | 21[c] | 6 | 4[d] | 10[e] | 14[f] |
| Vomiting | 0 | 15[d] | 4 | 4[f] | 7[d] | 11[e] |
| Diarrhea | 1 | 18[e] | 4 | 4[d] | 9[d] | 13[e] |

[a] Statistical comparisons were done by two-tailed Fisher's exact test between the nonsecretor (nonsec) group and either the secretor (Sec) group or the secretor group split by A, B, and O phenotypes: A Sec, B Sec, O Sec, non-A Sec (O or B Sec phenotype). A single secretor individual with diarrhea in the AB blood group was not included in the analysis of the A and B phenotypes.
[b] Individuals who had either vomiting or diarrhea.
[c] $P < 0.01$.
[d] $P < 0.05$.
[e] $P < 0.02$.
[f] $P < 0.005$.

The direct detection of viral human pathogens has become the most reliable manner for documenting viral contamination of shellfish, and sensitive methods are now available (4, 7, 23). Since the development of molecular methods, the inclusion of quality controls has become a priority and has been facilitated by the use of real-time RT-PCR, which allows sample-to-sample comparisons. Mengovirus (mengovirus strain $MC_0$, *Picornaviridae* family) was previously shown to be suitable as an external control for extraction efficiency on the basis of its structural characteristics and absence from environmental samples (7, 23). By using the different controls, the method allowed us to be confident that the concentrations expressed here correctly reflect the amount of virus present in the contaminated oysters. No adjustments for PCR amplification efficiency were made, since the results for the controls showed that the nucleic acid extracts had no effect on the performance of the rRT-PCR (data not shown). The last point that may influence the quantification of NoVs is sequence variations in the different NoV genotypes, which may lead to mismatches with the primers used in the real-time assay. This concern was offset by the results of previous studies that demonstrated the broad reactivity of the NoV-specific primers and probes used in the current study and by the fact that the predominant strain detected in the present study was GII.4, which corresponds to the strain used to build the standard curve (8). Expression of the sample concentration as a minimum and a maximum level (defined by taking the extraction efficiency into account) is one approach to estimation of the levels of NoV contamination. The large amount of oysters available allowed us to extract each sample at least four times to evaluate the reliability of our quantitative approach. The sample directly implicated in the outbreak showed the largest variation in NoV concentrations (up to 1,000-fold). This high level of variability may be explained by information from the producer, who later recognized that several batches of oysters had been mixed. After almost 1 month in the depuration tank, the geometric mean concentration had decreased only approximately twofold, although the level of variability within replicates was lower. This is another demonstration that virus contamination decreases very slowly within shellfish tissues and that although the use of depuration tanks is efficient in the case of bacterial contamination, it has little utility in addressing viral contamination (22, 27, 35). The levels of virus contamination measured in the two samples made up of shellfish entirely originating from the forbidden area were more homogeneous, suggesting frequent exposure to fecal contamination, possibly due to the close proximity to a major harbor.

The level of NoV contamination described in the present report was only slightly higher than the levels of shellfish viral contamination previously described for NoV outbreaks (20, 22, 36) and an HAV outbreak (7). Although quantitative information on the levels of NoV contamination in shellfish implicated in outbreaks is still limited, it is well above minimal infectious doses estimated from volunteers studies (2, 42).

Evidence accumulated over the past 6 years indicates that HBGAs serve as ligands for NoV infection (5, 6, 15, 17, 18, 25, 26, 39). However some studies showed discrepant results concerning the effect of either the ABO or the secretor phenotype, raising questions about the importance of HBGAs in norovirus infections (10, 12, 31, 32, 37). These discrepancies could be due to several factors, including an ill-defined carbohydrate specificity of the causative strain and poor or incomplete phenotyping of the affected individuals. The latter aspect is particularly relevant when variable or no associations with the ABO phenotype are reported. Since distinct strains show different specificities for HBGAs, the variable effects of the ABO phenotype are expected to occur in outbreaks caused by different NoV strains. Likewise, since the expression of ABH antigens in gut surface epithelial cells is strictly dependent upon the secretor status (33), a lack of information on the secretor phenotype makes it difficult to observe associations between HBGA expression and infection by NoVs. In the present study, we observed that the secretor phenotype was a risk factor associated with illness. That was particularly clear when individuals of the non-A subgroup were considered, which indicated that the A blood group antigen, which can be expressed on the digestive epithelial cells of secretors only, may have been a protective factor in this particular outbreak. Thus, polymorphisms at both the *FUT2* and the *ABO* loci controlled sensitivity to disease in this shellfish-related outbreak, confirming the importance of these polymorphisms in determining the susceptibility to NoV infection previously observed from either volunteer studies or community outbreaks (2, 5, 17, 24, 25). In this study, several strains (NoV, AiV, SaV) were detected either in stool samples or in shellfish. However, genetic sensitivity to NoV seems to be predominant, suggesting that NoVs induced most of the illnesses.

The comprehensive approach of our study, which consisted of the analysis of the implicated food, viral quantification, and HBGA typing of the consumers, is novel in the examination of food safety and provides a new approach to food safety analysis. The importance of the presence of different enteric viruses in stool and shellfish samples raises questions about which viruses were responsible for illness. The impact of HBGA expression in individuals with illness clearly implicates the GII NoVs as the predominant causative agents of the outbreak. When multiple enteric viruses contaminate a food product, genetic analysis of the affected individuals can complement the data collected for clinical and environmental samples and may provide important information for risk analysis and future food safety regulation.

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